

Pharmacokinetics of First-Line Antituberculosis Drugs in HIV-Infected Children with Tuberculosis Treated with Intermittent Regimens in India

Geetha Ramachandran,^a A. K. Hemanth Kumar,^a P. K. Bhavani,^a T. Kannan,^a S. Ramesh Kumar,^a N. Poorana Gangadevi,^a V. V. Banurekha,^a L. Sekar,^a N. Ravichandran,^b G. Mathevan,^c G. N. Sanjeeva,^d Rajeshwar Dayal,^e Soumya Swaminathan^a

National Institute for Research in Tuberculosis, ICMR, Chetpet, Chennai, India^a; Government Hospital of Thoracic Medicine, Chennai, India^b; Government Rajaji Hospital, Madurai, India^c; Indira Gandhi Institute of Child Health, Bengaluru, India^d; Sarojini Naidu Medical College, Agra, India^e

The objective of this report was to study the pharmacokinetics of rifampin (RMP), isoniazid (INH), and pyrazinamide (PZA) in HIV-infected children with tuberculosis (TB) treated with a thrice-weekly anti-TB regimen in the government program in India. Seventy-seven HIV-infected children with TB aged 1 to 15 years from six hospitals in India were recruited. During the intensive phase of TB treatment with directly observed administration of the drugs, a complete pharmacokinetic study was performed. Drug concentrations were measured by high-performance liquid chromatography. A multivariable regression analysis was done to explore the factors impacting drug levels and treatment outcomes. The proportions of children with subnormal peak concentrations (C_{max}) of RMP, INH, and PZA were 97%, 28%, and 33%, respectively. Children less than 5 years old had a lower median C_{max} and lower exposure (area under the time-concentration curve from 0 to 8 h [AUC_{0-8}]) of INH (C_{max} , 2.5 versus 5.1 $\mu\text{g/ml}$, respectively [$P = 0.016$]; AUC_{0-8} , 11.1 versus 22.0 $\mu\text{g/ml} \cdot \text{h}$, respectively [$P = 0.047$]) and PZA (C_{max} , 34.1 versus 42.3 $\mu\text{g/ml}$, respectively [$P = 0.055$]; AUC_{0-8} , 177.9 versus 221.7 $\mu\text{g/ml} \cdot \text{h}$, respectively [$P = 0.05$]) than those more than 5 years old. In children with unfavorable versus favorable outcomes, the median C_{max} of RMP (1.0 versus 2.8 $\mu\text{g/ml}$, respectively; $P = 0.002$) and PZA (31.9 versus 44.4 $\mu\text{g/ml}$, respectively; $P = 0.045$) were significantly lower. Among all factors studied, the PZA C_{max} influenced TB treatment outcome ($P = 0.011$; adjusted odds ratio, 1.094; 95% confidence interval, 1.021 to 1.173). A high proportion of children with HIV and TB had a subnormal RMP C_{max} . The PZA C_{max} significantly influenced treatment outcome. These findings have important clinical implications and emphasize that drug doses in HIV-infected children with TB have to be optimized.

Worldwide, tuberculosis (TB) is the most frequent coinfection in subjects with HIV-1 infection (1). A study from Zaire showed that the risk of TB in HIV-positive mothers was three times more than that in HIV-negative mothers (2). Infants born to HIV-infected mothers are susceptible to both HIV infection and TB. Several studies have demonstrated higher rates of childhood TB associated with increasing rates of disease in HIV-infected adults in the community (3, 4). High mortality rates have been observed with standard anti-TB treatment (ATT) regimens in HIV-infected children (5). A prospective cohort study observed that HIV-infected children with TB had a 6-fold higher mortality rate and decreased cure rates compared to those of HIV-negative children; HIV infection was the most important risk factor (6, 7). While HIV influences the TB treatment outcome in different ways, the inability to achieve and sustain therapeutic levels of anti-TB drugs (possibly due to malabsorption) can be a major factor in causing poor treatment outcomes. Malabsorption and decreased bioavailability of anti-TB drugs in adult patients with HIV and TB have been reported (8–11). A few studies have reported low anti-TB drug levels in HIV-infected children with TB (12, 13). However, these studies were conducted using the old TB treatment doses. After the World Health Organization (WHO) revision of doses for first-line anti-TB drugs, Thee et al. demonstrated that children less than 2 years of age achieved target concentrations of first-line anti-TB drugs according to the revised WHO recommendations (14).

Not much is known about the pharmacokinetics of first-line anti-TB drugs in HIV-infected children with TB in India. The Revised National TB Control Programme (RNTCP) uses stan-

dardized treatment regimens, with quality-ensured drugs provided through patient-wise boxes, tailored by weight bands in children (15). The entire duration of ATT is thrice weekly. While the majority of the TB control programs across the globe follow daily ATT, the Indian program continues to follow intermittent regimens. In the absence of pharmacokinetic data in HIV-infected children with TB treated with fully intermittent regimens in the RNTCP, we studied the pharmacokinetics of rifampin (RMP), isoniazid (INH), and pyrazinamide (PZA) and the factors influencing the drug pharmacokinetics and TB treatment outcomes.

MATERIALS AND METHODS

Patients. HIV-infected children aged 1 to 15 years with TB were recruited from the Institute of Child Health (Chennai, India), Government Hospital of Thoracic Medicine (Chennai), Kilpauk Medical College & Hospital

Received 18 September 2014 Returned for modification 18 October 2014

Accepted 29 November 2014

Accepted manuscript posted online 8 December 2014

Citation Ramachandran G, Kumar AKH, Bhavani PK, Kannan T, Kumar SR, Gangadevi NP, Banurekha VV, Sekar L, Ravichandran N, Mathevan G, Sanjeeva GN, Dayal R, Swaminathan S. 2015. Pharmacokinetics of first-line antituberculosis drugs in HIV-infected children with tuberculosis treated with intermittent regimens in India. *Antimicrob Agents Chemother* 59:1162–1167. doi:10.1128/AAC.04338-14.

Address correspondence to Soumya Swaminathan, soumyas@nirt.res.in.

Copyright © 2015, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.04338-14

(Chennai), Government Rajaji Hospital (Madurai, India), Indira Gandhi Institute of Child Health (Bengaluru, India), and Sarojini Naidu Medical College (Agra, India). All children were diagnosed with TB (pulmonary or extrapulmonary) by clinicians at the various sites. Diagnosis was based on clinical, radiographic, and histopathological evidence, and standard diagnostic criteria were followed. Diagnosis of HIV infection was based on an enzyme-linked immunosorbent assay (ELISA) results; in children <18 months of age, DNA-PCR testing was done to diagnose HIV infection. Children had received ATT according to the RNTCP guidelines for at least 2 weeks (six doses) with either of the following regimens: (i) a 6-month regimen with INH, RMP, PZA, and ethambutol (EMB) for 2 months followed by INH and RMP for the next 4 months (category I), or (ii) an 8-month regimen with streptomycin, INH, RMP, PZA, and EMB for 2 months followed by INH, RMP, PZA, and EMB for 1 month and INH, RMP, and EMB for the remaining 5 months (category II). The entire course of ATT was administered thrice weekly, which was the standard of care in India (15). The drug doses were based on body weight; doses were 10 mg/kg of body weight for RMP and INH, 30 to 35 mg/kg for PZA, 30 mg/kg for EMB, and 15 mg/kg for streptomycin. The drugs were administered by the parent/guardian of the child. Adherence to ATT was assessed by questioning the parent/guardian and reviewing the TB treatment card.

Individual drugs in blister packs were made available for the entire treatment duration in "patient-wise boxes," which are available in four different weight bands. Children eligible for antiretroviral treatment (ART) received treatment from the ART centers in the respective hospitals according to National AIDS Control Organization (NACO) guidelines. This consisted of efavirenz and lamivudine with either zidovudine or stavudine or tenofovir or abacavir. Parents/guardians gave informed written consent; children more than 7 years old gave their assent. The study was approved by the institutional ethics committee of each study site.

Clinical assessment. A clinical examination and documentation of baseline demographic data were performed for each eligible child. The clinical history was collected from RNTCP records of the children. Adherence to ATT was assessed by questioning the parents/guardians and reviewing the treatment cards. Anthropometric data (body weight, height, head circumference, and mid-arm circumference) were measured on the pharmacokinetic study day. The clinical management of patients was done by site physicians.

Determination of phenotypic INH acetylase status. Two milliliters of saliva was collected 5 h after the administration of INH syrup at a dose of 2.5 mg/kg of body weight. Salivary INH levels were assessed by high-performance liquid chromatography (HPLC), and an INH concentration of 0.3 µg/ml or less was indicative of a rapid acetylase status (16, 17).

Conduct of pharmacokinetic study. The study was conducted at the inpatient wards in the hospitals after children had received at least six doses of anti-TB drugs (i.e., at steady state). On the study day, serial blood samples (2 ml each) at predosing and at 2, 4, 6, and 8 h after drug intake were collected. The anti-TB drugs were administered under direct observation after an overnight fast. Older children were asked to swallow the tablets with water, while younger children who could not swallow tablets received the tablets in powdered form mixed in a small quantity of water and were asked to swallow the entire contents.

Drug estimations. Plasma INH, PZA, and RMP concentrations were determined by HPLC using validated methods (17, 18). The between- and within-run variations for all the drugs were less than 10%. The lower limits of quantification for RMP, INH, and PZA were 0.25 µg/ml, 0.25 µg/ml, and 1.25 µg/ml, respectively.

Calculation of pharmacokinetic variables. Peak concentrations (C_{max} values) were determined by visual inspection of the data. The linear trapezoidal rule was used to compute exposures, or the areas under the time-concentration curve from 0 to 8 h (AUC_{0-8} values).

Assessment of nutritional status. The Z scores for weight and height were computed based on each child's age and gender using the EPI-NUT

TABLE 1 Demographic and clinical features of children with TB and HIV^a

Characteristic	Data ^b
Age (yr)	9.0 (6.5–11.0)
Male	50 (65)
Body wt (kg)	17.0 (14.1–22.5)
Nutritional status ^c	
HAZ	−3.0 (−4.1 to −2.0)
WAZ	−2.7 (−3.4 to −1.9)
WHZ ^d	−1.1 (−1.7 to 0.02)
Mid-arm circumference (cm)	14.0 (12.0–16.0)
Head circumference (cm)	48.0 (43.0–50.0)
Thrice-weekly dose (mg/kg)	
RMP	10.0 (7.5–11.3)
INH	10.0 (7.5–11.3)
PZA	33.0 (27.8–37.5)
Duration of ATT (mo)	1.0 (0.5–1.0)
Regimen	
Category I	66 (86)
Category II	11 (14)
Type of TB	
Pulmonary	49 (64)
Extrapulmonary	28 (36)
Rapid acetylators of INH	25 (30)
CD4 cell count (cells/mm ³)	359.0 (192.5–722.5)
On antiretroviral treatment	45 (60)

^a $n = 77$.

^b Data presented as no. (%) or median (interquartile range).

^c HAZ, height-for-age Z score; WAZ, weight-for-age Z score; WHZ, weight-for-height Z score.

^d Values from 56 children.

component of the EPI-INFO 2002 software package (version 3.4.3) from the CDC (based on National Center for Health Statistics reference median values) (19).

Treatment and follow-up. Children continued ATT per the program guidelines. The treatment outcomes were noted from the card; cured/treatment completion was considered a favorable outcome, while failure, death, and default were unfavorable outcomes.

Sample size. Based on the findings of Palme et al. (20), who, when considering treatment outcome as the primary endpoint, observed a cure rate of 58% among HIV-positive patients with TB, and using a 95% confidence interval (CI), 80% power, and oversampling of 5% during follow-up, the sample size was calculated to be 73 children.

Statistical evaluation. The analysis of data was performed using SPSS, version 14.0. The values are expressed medians with interquartile ranges. The Shapiro-Wilks test showed that the pharmacokinetic data were not normally distributed. The Mann-Whitney U test was used to compare the C_{max} and AUC_{0-8} between the different groups of children. Subnormal C_{max} values were defined for RMP as <8 µg/ml, for INH as <3 µg/ml, and for PZA as <35 µg/ml (21). A comparison of the proportions between the groups was done using the Z proportion test. Pearson's correlation test was performed to determine the correlation between the C_{max} of the drugs and the doses in mg/kg of body weight. Univariate and multivariate regression analyses by the stepwise method were performed to determine the factors that influenced the C_{max} and AUC_{0-8} of RMP, INH, and PZA. A multiple logistic regression analysis by a backward deletion algorithm was used to identify the factors that influenced the treatment outcome. A *P* value of <0.05 was considered statistically significant.

TABLE 2 Pharmacokinetics of RMP, INH, and PZA in HIV-infected children with TB^a

Variable	No. of patients	Pharmacokinetic data ^b for:					
		RMP		INH		PZA	
		C_{\max} ($\mu\text{g/ml}$)	AUC_{0-8} ($\mu\text{g/ml} \cdot \text{h}$)	C_{\max} ($\mu\text{g/ml}$)	AUC_{0-8} ($\mu\text{g/ml} \cdot \text{h}$)	C_{\max} ($\mu\text{g/ml}$)	AUC_{0-8} ($\mu\text{g/ml} \cdot \text{h}$)
Age							
≤5 yr	12	2.4 (1.1–4.1)	9.5 (3.8–13.2)	2.5 (2.9–4.3)	11.1 (7.1–16.1)	34.1 (21.4–40.9)	177.9 (116.8–232.9)
>5 yr	65	2.7 (1.4–4.6)	10.9 (6.5–19.8)	5.1 (3.1–7.5) ^c	22.0 (11.8–31.2) ^c	42.3 (32.3–51.4) ^d	221.7 (177.8–283.4) ^c
Sex							
Male	50	2.7 (1.1–4.5)	10.4 (6.1–18.8)	5.5 (3.3–8.4)	23.5 (13.9–40.0)	42.6 (33.3–57.3)	227.4 (189.4–287.2)
Female	27	2.5 (1.3–4.6)	10.5 (5.9–18.8)	3.7 (2.6–7.0)	15.1 (9.6–27.0) ^c	40.3 (31.1–47.8)	218.1 (169.4–271.8)
Nutritional status							
Normal	18	3.9 (1.7–4.5)	15.4 (5.6–23.4)	5.3 (3.3–7.3)	22.7 (12.4–31.4)	34.5 (31.5–43.9)	201.2 (166.9–264.3)
Stunted	59	2.4 (1.1–4.4)	10.1 (6.1–17.6)	4.6 (2.5–7.3)	19.5 (10.0–30.8)	43.2 (31.8–49.1)	226.8 (177.8–279.1)
Weight							
Normal	21	3.3 (1.1–4.7)	9.9 (3.6–22.0)	5.4 (3.1–7.5)	22.1 (11.6–31.5)	40.6 (29.4–46.8)	211.8 (157.0–266.4)
Underweight	56	2.5 (1.3–4.2)	10.9 (6.7–17.7)	4.4 (2.7–7.2)	19.2 (10.6–30.7)	41.3 (31.4–48.6)	224.6 (175.5–284.9)
Weight status							
Normal	41	2.3 (1.2–4.7)	9.7 (5.7–17.6)	5.3 (3.2–7.8)	22.3 (12.4–34.5)	43.2 (33.9–49.1)	218.1 (182.8–282.8)
Wasted ^e	15	3.4 (1.9–4.4)	11.5 (6.5–19.8)	3.3 (2.7–5.3)	12.2 (10.5–26.6)	34.5 (28.5–42.6)	186.4 (169.4–247.9)
BMI							
<18.5 kg/m ²	70	2.7 (1.3–4.6)	10.8 (6.3–19.1)	5.3 (2.5–7.1)	21.9 (10.5–26.9)	41.1 (31.2–48.0)	218.1 (171.3–272.6)
≥18.5 kg/m ²	7	2.2 (1.1–4.1)	7.2 (2.7–10.2)	4.1 (2.9–7.5)	19.0 (10.7–32.3)	49.6 (39.0–62.2)	282.3 (191.4–331.0)
Serum albumin level							
Within normal range	20	3.4 (1.6–5.3)	15.4 (8.9–22.7)	4.6 (2.7–7.2)	19.5 (10.8–29.3)	44.6 (28.5–55.5)	255.4 (168.1–288.5)
Below normal range	57	2.4 (1.2–4.4)	9.5 (5.0–17.6)	6.7 (4.1–7.6)	33.3 (15.8–35.2)	41.1 (32.4–47.3)	216.0 (174.4–260.7)
Outcome							
Favorable	54	2.8 (1.8–4.7)	10.6 (7.0–17.6)	4.3 (2.8–7.3)	17.5 (10.7–30.1)	44.4 (40.3–52.2)	248.1 (207.5–284.9)
Unfavorable	18	1.0 (0.2–3.1) ^c	4.7 (2.2–17.2) ^c	5.1 (2.9–7.2)	23.4 (10.9–32.7)	31.9 (26.4–38.7) ^c	177.3 (151.8–217.0) ^c
ART							
Without	28	2.6 (1.7–5.2)	11.1 (6.9–22.0)	4.6 (3.1–6.9)	21.1 (12.0–30.1)	44.4 (34.0–54.9)	226.8 (201.2–283.9)
With	49	2.8 (1.1–4.3)	10.6 (4.2–17.6)	4.8 (2.4–7.6)	18.7 (8.1–31.3)	41.0 (27.8–45.7)	209.6 (167.5–259.1)
Acetylator status							
Slow	52			5.4 (3.2–7.7)	23.9 (14.2–33.6)		
Rapid	25			3.2 (2.0–4.3) ^c	11.1 (7.4–15.1) ^c		

^a $n = 77$.^b Values are reported as medians (interquartile ranges). C_{\max} , peak concentration; AUC_{0-8} , exposure.^c $P < 0.05$.^d $P = 0.055$.^e Z scores could not be calculated for 21 children.

RESULTS

A total of 77 children coinfecting with HIV and TB took part in the study. Their demographic and clinical features are given in Table 1. Overall, 86% of the children received the 6-month category I treatment, and 30% of these children were INH rapid acetylators. While 64% of the children had pulmonary TB, extrapulmonary forms included cervical and axillary lymph node TB ($n = 12$ children), hilar adenitis ($n = 8$), mediastinal adenitis ($n = 4$), TB meningitis ($n = 2$), TB of the spine ($n = 1$), and TB of the abdomen ($n = 1$).

C_{\max} and AUC_{0-8} of drugs. C_{\max} and AUC_{0-8} data for RMP, INH, and PZA in the different groups of children are given in Table 2. The differences in the C_{\max} and AUC_{0-8} between children less than 5 years and those more than 5 years of age were statistically significant for INH and PZA but not for RMP; however,

children more than 5 years old had higher RMP C_{\max} and AUC_{0-8} values. Children belonging to these two groups did not differ in the doses received (RMP and INH, 11.0 and 9.7 mg/kg, respectively [$P = 0.28$]; PZA, 39.0 and 32.4 mg/kg, respectively [$P = 0.24$]).

Females had lower INH C_{\max} and AUC_{0-8} values than males, and the difference was statistically significant for the AUC_{0-8} . Slow acetylators had significantly higher INH C_{\max} and AUC_{0-8} values than the rapid acetylators ($P < 0.01$).

Children with unfavorable outcomes had significantly lower median C_{\max} and AUC_{0-8} values for RMP (2.5 versus 4.4 $\mu\text{g/ml}$, respectively [$P < 0.001$]; 12.3 versus 17.9 $\mu\text{g/ml} \cdot \text{h}$, respectively [$P = 0.008$]) and PZA (31.3 versus 44.4 $\mu\text{g/ml}$, respectively [$P = 0.002$]; 173.2 versus 250.2 $\mu\text{g/ml} \cdot \text{h}$, respectively [$P = 0.003$]) than those who had favorable outcomes (Fig. 1).

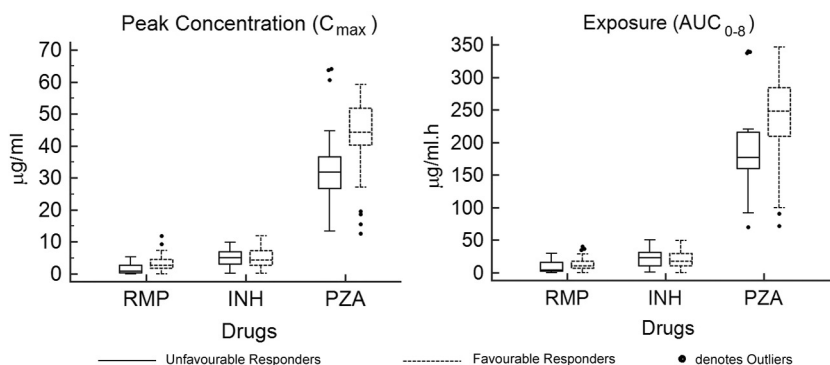


FIG 1 Median RMP, INH, and PZA peak concentrations and exposure in children with favorable and unfavorable responses to anti-TB treatment.

Subtherapeutic C_{max} . Subtherapeutic C_{max} values for RMP, INH, and PZA were observed in 97%, 28%, and 33% children, respectively. A higher proportion of children with a subtherapeutic INH C_{max} were rapid acetylators than those who were slow acetylators (46% versus 21%, respectively; $P = 0.03$). The proportions of children with subtherapeutic INH and PZA C_{max} values were higher in children less than 5 years old than those more than 5 years old (INH, 55% versus 23%, respectively [$P = 0.03$]; PZA, 63% versus 29%, respectively [$P = 0.06$]).

Factors influencing C_{max} and AUC_{0-8} values of RMP, INH, and PZA. The factors included for analysis were age, sex, height-for-age Z score (HAZ), weight-for-age Z score (WAZ), weight-for-height Z score (WHZ), serum albumin level, and INH acetylator status. The univariate analysis was done first, and only those variables that gave a P value of <0.1 were further tested by a multivariate regression analysis by the stepwise method. Serum albumin levels had a significant influence on the AUC_{0-8} of RMP, and acetylator status significantly influenced the INH C_{max} and AUC_{0-8} values (Table 3).

A positive significant correlation was observed among the C_{max} values of RMP, INH, and PZA with the respective doses. The r and P values were 0.162 and 0.043 for RMP, 0.387 and <0.001 for INH, and 0.353 and <0.001 for PZA, respectively.

TB treatment outcome. TB treatment outcomes were known for 72 children; five children were lost during follow-up. An unfavorable outcome was observed in 18 children (8 deaths and 10 treatment failures). Univariate logistic regression analysis was performed to identify factors (age, body mass index [BMI], sex,

HAZ, WAZ, WHZ, serum albumin level, INH acetylator status, RMP C_{max} , INH C_{max} , and PZA C_{max}) that had a significant impact on the treatment outcome. The RMP C_{max} and the PZA C_{max} had significant impacts ($P < 0.05$; Fig. 2). Further, those factors with a P value of <0.1 in univariate analysis were taken for logistic regression analysis by the stepwise method. It was observed that the PZA C_{max} ($P = 0.011$; adjusted odds ratio, 1.094; 95% CI, 1.021 to 1.173) significantly influenced the treatment outcome.

DISCUSSION

This prospective study has provided pharmacokinetic data of key first-line anti-TB drugs in HIV-infected children with TB. Similar to previous studies, we observed lower INH and PZA C_{max} values in younger children (15, 22, 23). We did not observe any difference in RMP concentrations between younger and older children, perhaps because the RMP C_{max} was low in a majority of the children across all ages. Furthermore, the proportions of children with subtherapeutic INH and PZA C_{max} values were higher in children less than 5 years old. This is despite the similar drug dosages used for children less than and more than 5 years of age. Age-dependent elimination of INH has been demonstrated, with younger children eliminating the drug more rapidly than older children (15, 22). This has been attributed to the relatively larger liver size in proportion to total body weight in younger children (24). Age has a significant impact on drug concentrations and should be considered while making dosing recommendations in children.

We compared pharmacokinetic parameters in relation to malnutrition. Although children with stunting, who were underweight, or who had a BMI and a serum albumin level below the normal ranges had lower drug concentrations than did well-nourished children, the differences did not reach significance. While some studies have reported malnutrition to be a significant predictor of anti-TB drug concentrations (14, 15, 22, 23), there are few studies which did not find a major impact of malnutrition on drug concentrations (25–27).

Irrespective of age and whether the patients are adults or children, the RMP concentrations are the most affected and are subnormal in a high proportion of HIV-infected patients. However, treatment outcomes are often satisfactory. The relationship between plasma drug concentrations and the outcome of TB treatment has been a matter of debate and likely involves the interplay of multiple factors. Treatment outcome is driven by multiple factors, such as the strain involved, virulence, MIC, duration of in-

TABLE 3 Multiple regression analysis showing factors significantly influencing peak concentration and exposure of RMP, INH, and PZA

Factor ^a	Regression data		
	β	95% CI	P
RMP			
AUC_{0-8} ($\mu\text{g/ml} \cdot \text{h}$)			
Serum albumin level	5.495	0.350–10.641	0.037
INH			
C_{max} ($\mu\text{g/ml}$)			
Acetylator status	2.116	0.313–3.919	0.022
AUC_{0-8} ($\mu\text{g/ml} \cdot \text{h}$)			
Acetylator status	13.443	5.084–21.801	0.002

^a Age, acetylator status (for INH), serum albumin level, HAZ, WAZ, and WHZ were used in the multiple linear regression analysis by the stepwise method.

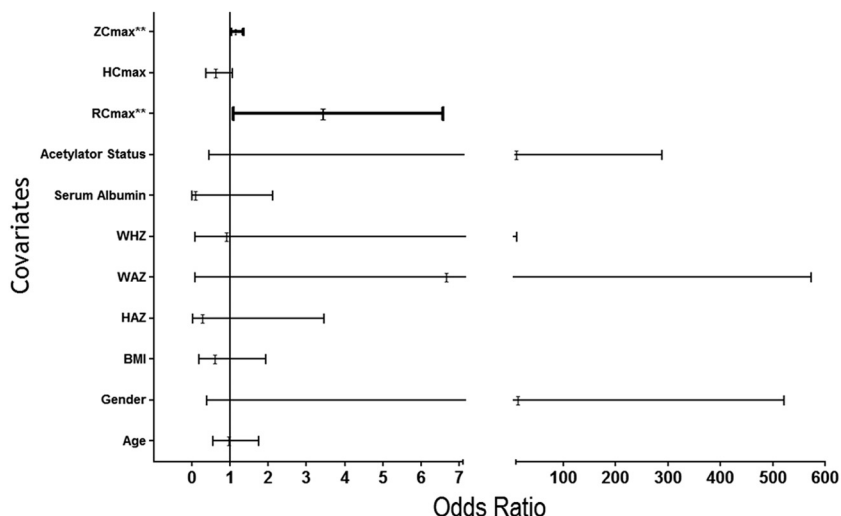


FIG 2 Unadjusted odds ratio of factors influencing TB treatment outcome. **, P value < 0.05. RCmax, C_{\max} of rifampin; HCmax, C_{\max} of isoniazid; ZCmax, C_{\max} of pyrazinamide; C_{\max} , peak concentration; BMI, body mass index; WAZ, weight-for-age Z score denoting underweight; HAZ, height-for-age Z score; WHZ, weight-for-height Z score.

fection, extent of disease, drug concentrations, immune status, nutritional status, and gene polymorphisms. However, persistently low concentrations can encourage the emergence of drug-resistant *Mycobacterium tuberculosis*. As treatment progresses, such patients are likely to experience an increased risk of developing resistance, manifesting as delayed smear conversion, treatment failure, or relapse. Hence, maintaining optimal drug concentrations is important and ensures that doses are adequate to achieve the required bactericidal effect.

Importantly, we examined the relationship between drug pharmacokinetics and TB treatment outcomes in children with TB and HIV. After controlling for all factors, we observed that the PZA C_{\max} significantly influenced treatment outcome, a finding similar to those in published reports (28, 29). Burhan et al. observed an association of low PZA concentrations with a less favorable bacteriological response in adult Indonesian TB patients (28). In a study from Botswana in an HIV-infected cohort of adult TB patients, a low PZA C_{\max} was associated with poor treatment outcome (29). PZA is a key drug that kills intracellular organisms and is given for only 2 months. These studies highlight the need to treat children with adequate drug doses. A study from Virginia in adult TB patients reported that slow responders to therapy had subtherapeutic concentrations of RMP, INH, and ethambutol (30). Using a hollow-fiber system model of TB, Pasipanodya et al. concluded that low AUCs of RMP, INH, and PZA were predictive of clinical outcomes in TB patients (31). Our study finding raises an important question: were these children underdosed, hence the low drug concentrations? This assumes significance in light of the study findings of Thee et al., who observed that children less than 2 years of age achieved target drug concentrations when treated with the revised WHO-recommended doses (14).

A study limitation was that blood concentrations were determined only up to 8 h postdosing, and this might have affected the PZA AUC. In conclusion, this study, done in a fairly large group of HIV-infected children with TB, has provided evidence that the concentrations of first-line anti-TB drugs are subnormal in a significant proportion of them, and this appears to influence treat-

ment outcomes. This also is likely due to low drug doses that were used intermittently. The finding of PZA playing a key role in TB treatment outcomes needs further attention. Improving outcomes in HIV-infected children with TB requires multiple interventions; an important one will be to optimize drug dosing.

ACKNOWLEDGMENTS

We thank the children who participated in the study, K. Nandagopal (ART medical officer, Kilpauk Medical College and Hospital, Chennai) for assistance with patient recruitment, V. Sudha for drug estimations, S. Venkatesh for counseling and follow-up of patients, and NIRT clinic nurses for blood collection.

This study was funded by the Pediatric HIV Task Force of the Indian Council of Medical Research, New Delhi.

We declare no conflicts of interest.

REFERENCES

- Dye C, Scheele S, Dolin P, Pathana V, Ravignone MC. 1999. Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. WHO Global Surveillance and Monitoring Project. *JAMA* 282:677–686.
- Braun MM, Badi N, Ryder RW, Baende E, Mukadi Y, Nsuami M, Matela B, Williams JC, Kaboto M, Heyward W. 1991. A retrospective cohort study of the risk of tuberculosis among women of child bearing age with HIV infection in Zaire. *Am Rev Respir Dis* 143:501–504. <http://dx.doi.org/10.1164/ajrccm/143.3.501>.
- Harries AD. 1990. Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet* 335:387–390. [http://dx.doi.org/10.1016/0140-6736\(90\)90216-R](http://dx.doi.org/10.1016/0140-6736(90)90216-R).
- Jones D, Malecki J, Bigler W, Witte JJ, Oxtoby MJ. 1999. Pediatric tuberculosis and human immunodeficiency virus infection in Palm Beach County, Florida. *Am J Dis Child* 146:1166–1170.
- Swaminathan S. 2004. Tuberculosis in HIV-infected children. *Paediatr Respir Rev* 5:225–230. <http://dx.doi.org/10.1016/j.prrv.2004.04.006>.
- Jeena PM, Pillay P, Pillay T, Coovadia HM. 2002. Impact of HIV-1 coinfection on presentation and hospital-related mortality in children with culture proven pulmonary tuberculosis in Durban, South Africa. *Int J Tuberc Lung Dis* 6:672–678.
- Rekha B, Swaminathan S. 2007. Childhood tuberculosis: global epidemiology and the impact of HIV. *Paediatr Respir Rev* 8:99–106. <http://dx.doi.org/10.1016/j.prrv.2007.04.010>.
- Gurumurthy P, Ramachandran G, Hemanth Kumar AK, Rajasekaran S,

- Padmapriyadarsini C, Soumya Swaminathan Bhagavathy S, Venkatesan P, Sekar L, Mahilmaran A, Ravichandran N, Paramesh P. 2004. Decreased bioavailability of rifampin and other anti-TB drugs in patients with advanced HIV disease. *Antimicrob Agents Chemother* 48:4473–4475. <http://dx.doi.org/10.1128/AAC.48.11.4473-4475.2004>.
9. Sahai J, Gallicano K, Swick L, Tailor S, Garber G, Sequin I, Oliveras L, Walker S, Rachlis A, Cameron DW. 1997. Reduced plasma concentrations of antituberculous drugs in patients with HIV infection. *Ann Intern Med* 127:289–293. <http://dx.doi.org/10.7326/0003-4819-127-4-199708150-00006>.
 10. Peloquin CA, MacPhee AA, Berning SE. 1993. Malabsorption of antimycobacterial medications by a patient with AIDS. *N Engl J Med* 329:1122–1123. <http://dx.doi.org/10.1056/NEJM199310073291513>.
 11. Berning SE, Huitt GA, Iseman MD, Peloquin CA. 1992. Malabsorption of antituberculosis medications by a patient with AIDS. *N Engl J Med* 327:1817–1818. <http://dx.doi.org/10.1056/NEJM199212173272514>.
 12. Graham SM, Bell DJ, Nyirongo S, Hartkoorn R, Ward SA, Molyneux EM. 2006. Low levels of pyrazinamide and ethambutol in children with tuberculosis and impact of age, nutritional status, and human immunodeficiency virus infection. *Antimicrob Agents Chemother* 50:407–413. <http://dx.doi.org/10.1128/AAC.50.2.407-413.2006>.
 13. Schaaf HS, Willemsse M, Cilliers K, Labadarios D, Maritz JS, Hussey GD, McIlleron H, Smith PJ, Donald PR. 2009. Rifampin pharmacokinetics in children, with and without human immunodeficiency virus infection, hospitalized for the management of severe forms of tuberculosis. *BMC Med* 7:19–29. <http://dx.doi.org/10.1186/1741-7015-7-19>.
 14. Thee S, Seddon JA, Donald PR, Seifart SI, Werely CJ, Hesselning AC, Rosenkranz B, Roll S, Magdorf K, Schaaf HS. 2011. Pharmacokinetics of isoniazid, rifampin, and pyrazinamide in children younger than two years of age with tuberculosis: evidence for implementation of revised World Health Organization recommendations. *Antimicrob Agents Chemother* 55:5560–5567. <http://dx.doi.org/10.1128/AAC.05429-11>.
 15. Revised National Tuberculosis Control Programme. Technical and operational guidelines for tuberculosis control, October 2005. Ministry of Health and Family Welfare, New Delhi, India. <http://tbcindia.nic.in/pdfs/Technical%20&%20Operational%20guidelines%20for%20TB%20Control.pdf>.
 16. Sarma GR, Kailasam S, Datta M, Loganathan GK, Rahman F, Narayana ASL. 1990. Classification of children as slow or rapid acetylators based on concentrations of isoniazid in saliva following oral administration of body weight and surface area: related dosages of the drug. *Indian Pediatr* 27:134–142.
 17. Kumar AKH, Sudha V, Ramachandran G. 2012. Simple and rapid liquid chromatography method for simultaneous determination of isoniazid and pyrazinamide in plasma. *SAARC J TB Lung Dis HIV/AIDS* 9:13–18. <http://dx.doi.org/10.3126/saarctb.v9i1.6960>.
 18. Kumar AKH, Chandra I, Geetha R, Chelvi KS, Lalitha V, Prema G. 2004. A validated high-performance liquid chromatography method for the determination of rifampicin and desacetyl rifampicin in plasma and urine. *Indian J Pharmacol* 36:231–233.
 19. World Health Organization. 2011. Physical status: the use and interpretation of anthropometry. Report of a WHO expert committee. Technical report series no. 854. World Health Organization, Geneva, Switzerland. http://www.who.int/childgrowth/publications/physical_status/en/index.html.
 20. Palme IB, Gudetta B, Bruchfeld J, Muhe L, Giesecke J. 2002. Impact of human immunodeficiency virus 1 infection on clinical presentation, treatment outcome and survival in a cohort of Ethiopian children with tuberculosis. *Pediatr Infect Dis J* 21:1053–1061. <http://dx.doi.org/10.1097/00006454-200211000-00016>.
 21. Alsultan A, Peloquin CA. 2014. Therapeutic drug monitoring in the treatment of tuberculosis: an update. *Drugs* 74:839–854. <http://dx.doi.org/10.1007/s40265-014-0222-8>.
 22. McIlleron H, Willemsse M, Werely CJ, Hussey GD, Schaaf HS, Smith PJ, Donald PR. 2009. Isoniazid plasma concentrations in a cohort of South African children with tuberculosis: implications for international pediatric dosing guidelines. *Clin Infect Dis* 48:1547–1553. <http://dx.doi.org/10.1086/598192>.
 23. Ramachandran G, Hemanth Kumar AK, Bhavani PK, Poorana Gangadevi N, Sekar L, Vijayasekaran D, Banu Rekha VV, Ramesh Kumar S, Ravichandran N, Mathevan G, Soumya Swaminathan. 2013. Age, nutritional status and isoniazid acetylator status influence pharmacokinetics of anti-TB drugs in children. *Int J Tuberc Lung Dis* 17:800–806. <http://dx.doi.org/10.5588/ijtld.12.0628>.
 24. Schaaf HS, Parkin DP, Seifart HI, Werely J, Hesselning PB, van Helden PD, Maritz JS, Donald PR. 2005. Isoniazid pharmacokinetics in children treated for respiratory tuberculosis. *Arch Dis Child* 90:614–618. <http://dx.doi.org/10.1136/adc.2004.052175>.
 25. Seifart HI, Donald PR, de Villiers JN, Parkin DP, Jaarsveld PP. 1995. Isoniazid elimination kinetics in children with protein-energy malnutrition treated for tuberculous meningitis with a four-component antimicrobial regimen. *Ann Trop Paediatr* 15:249–254.
 26. Seth V, Beotra A, Bagga A, Seth SD. 1992. Drug therapy in malnutrition. *Indian Pediatr* 29:1341–1346.
 27. Seth V, Beotra A, Seth SD, Semwal OP, Kabra S, Jain Y, Mukhopadhyaya S. 1993. Serum concentrations of rifampicin and isoniazid in tuberculosis. *Indian Pediatr* 30:1091–1098.
 28. Burhan E, Ruesen C, Ruslami R, Ginanjar A, Mangunnegoro H, Ascobat P, Donders R, van Crevel R, Aarnoutse R. 2013. Isoniazid, rifampin and pyrazinamide plasma concentrations in relation to treatment response in Indonesian pulmonary tuberculosis patients. *Antimicrob Agents Chemother* 57:3614–3619. <http://dx.doi.org/10.1128/AAC.02468-12>.
 29. Chideya S, Winston CA, Peloquin CA, Bradford WZ, Hopewell PC, Wells CD, Reingold AL, Kenyon TA, Moeti TL, Tappero JW. 2009. Isoniazid, rifampin, ethambutol, and pyrazinamide pharmacokinetics and treatment outcomes among predominantly HIV-infected cohort of adults with tuberculosis from Botswana. *Clin Infect Dis* 48:1685–1694. <http://dx.doi.org/10.1086/599040>.
 30. Heysell SK, Moore JL, Keller SJ, Houpt ER. 2010. Therapeutic drug monitoring for slow response to tuberculosis treatment in a state control program, Virginia, USA. *Emerg Infect Dis* 16:1546–1553. <http://dx.doi.org/10.3201/eid1610.100374>.
 31. Pasipanodya JG, McIlleron H, Burger A, Wash PA, Gumbo T. 2013. Serum drug concentrations predictive of pulmonary tuberculosis outcomes. *J Infect Dis* 208:1464–1473. <http://dx.doi.org/10.1093/infdis/jit352>.